

Replication of RSV subgroup A mutants in the lungs of
BALB/c mice correlates with replication in the nasopharynx
of seronegative chimpanzees

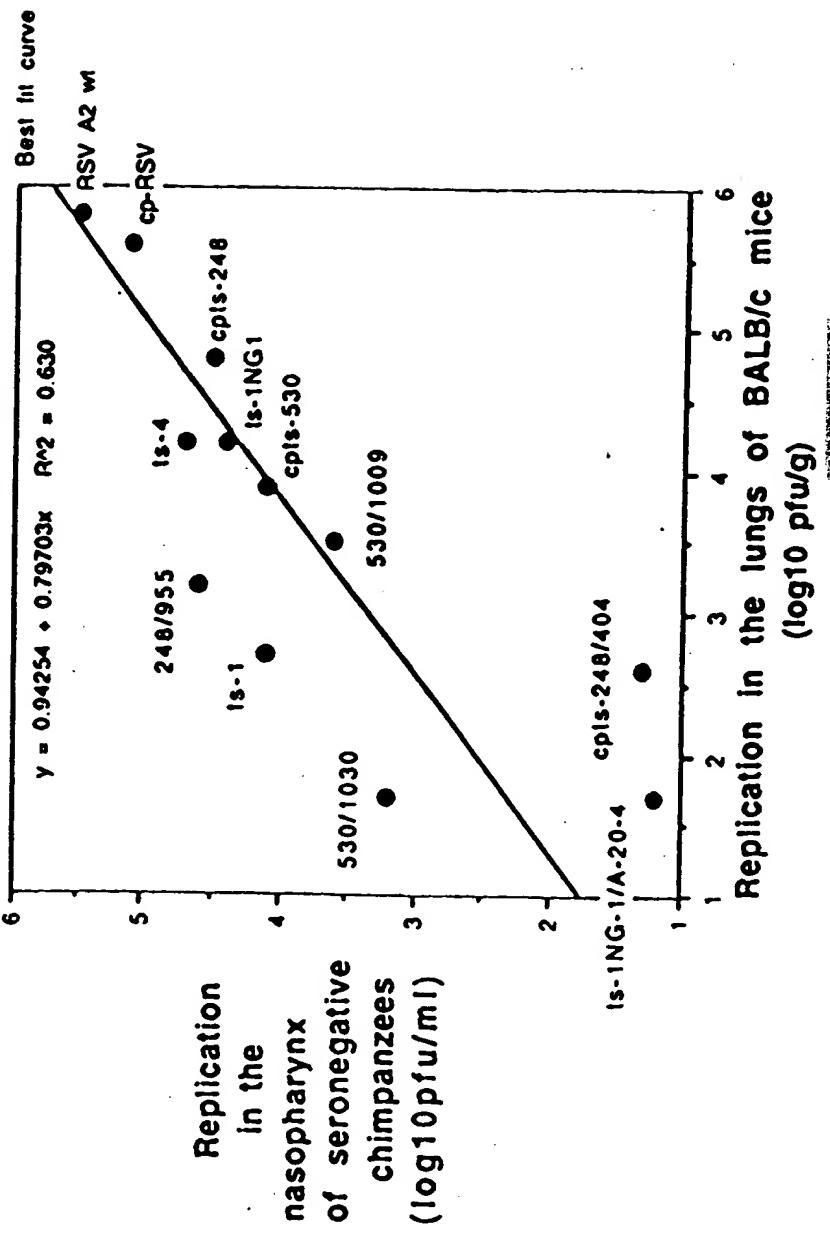


FIG. 1

24

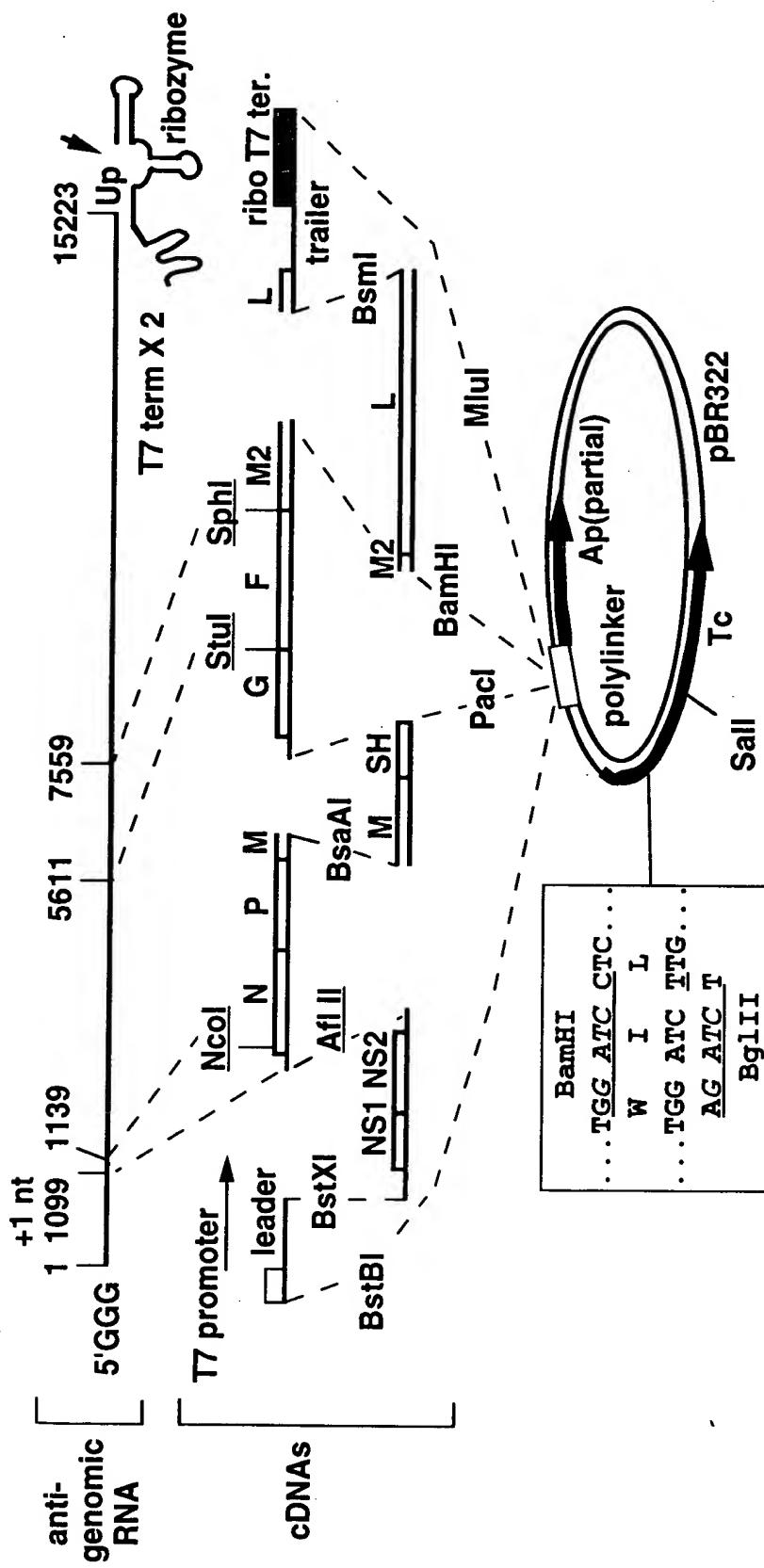


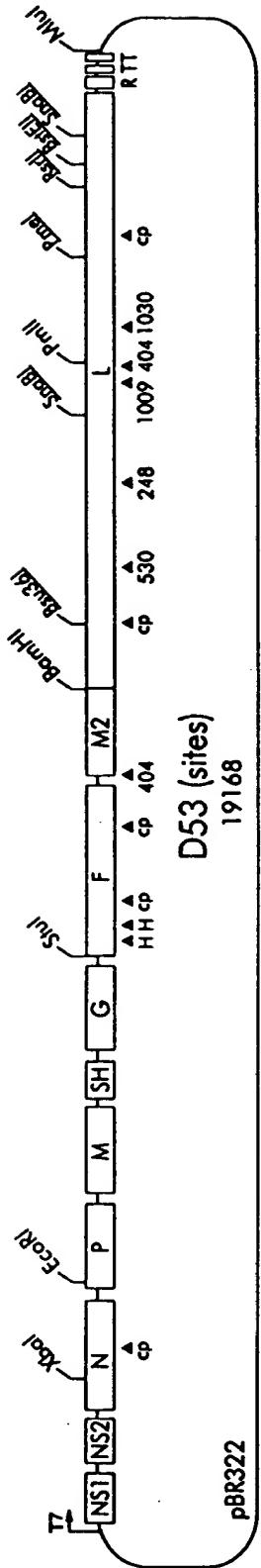
Fig. 2

FIG. 3

NS2	1099	GE	NS2/N intergenic	N GS	1139	N orf
ÅÅÅÅ	ÜUÅÅGGAGAGAUAUÅLGÅUÅGÅÅGAÜ		[GGGGCÅÅÅÜ]	ÅCÅÅÅGÅUÅGGC		
A				CC		
AFIII					NcoI	
G GE	5611		G/F intergenic			
ÜUÅÅÅÅ	ÇLAUAUÜAÜCÅÅCÅÅÅGCCÅUGACCAACÜUÅÅÅCÅGLÅUÅCÅÅÅÅÜA		[G G]			
			StuI			
F GE	7559		F/M2 intergenic		M2 GS	
ÅÅÅ	ÇÅCÅÅSUQÅLUGCCÅGÅUÅACUÜÅCÅÜCUOSUÅÅÅÅÅÜGÅÅÅÅCÜ	C	[GG]			
			SphI			

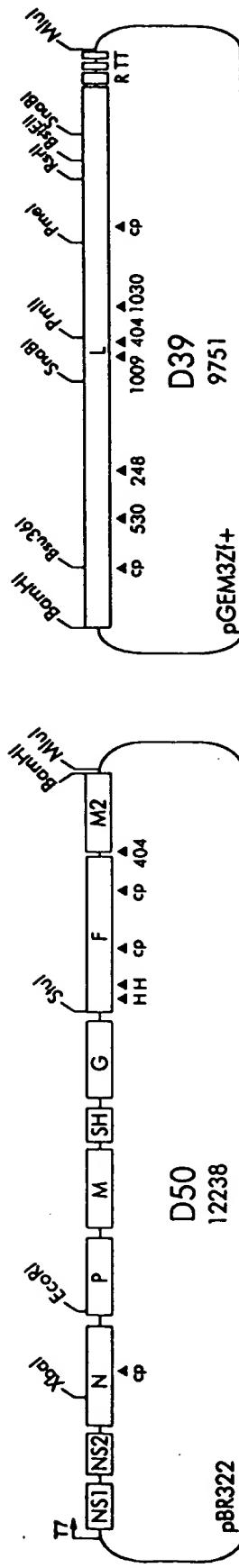
pBR322

1000
900
800
700
600
500
400
300
200
100
0



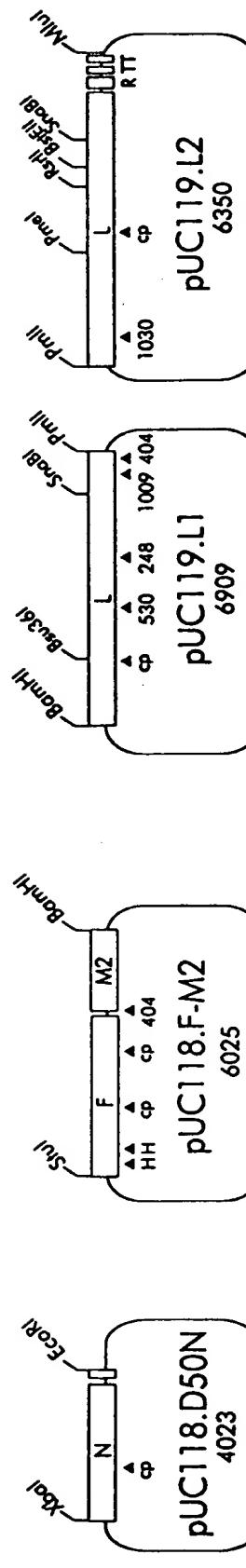
D53 (sites)
19168

pBR322



D39
9751

pGEM3Zf+



pUC119.L2
6350

pUC118.D50N
4023

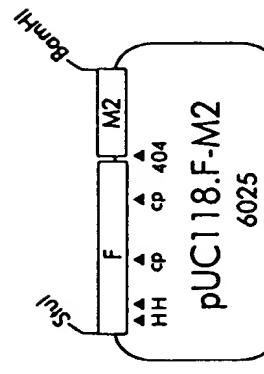


FIG. 4

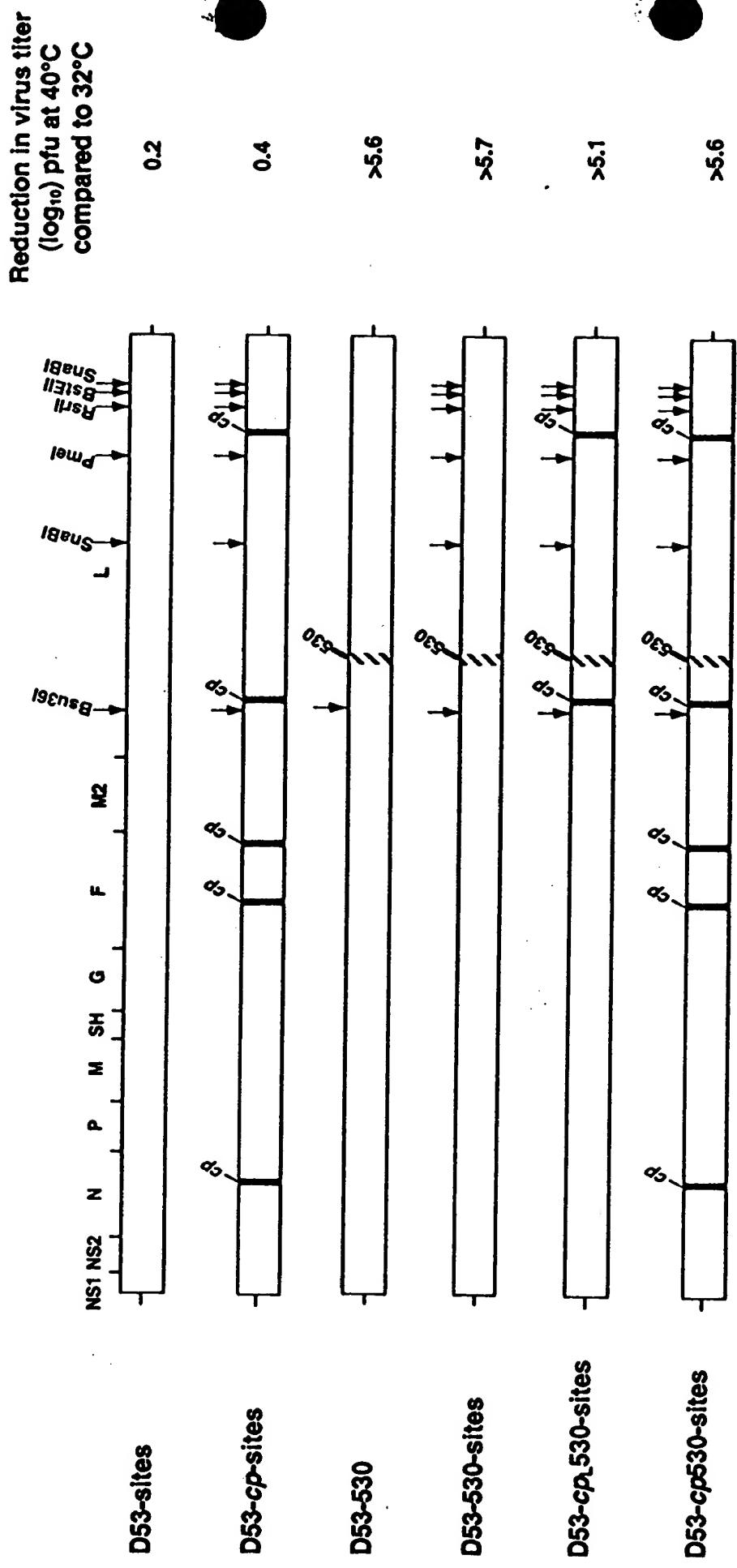


FIG. 5

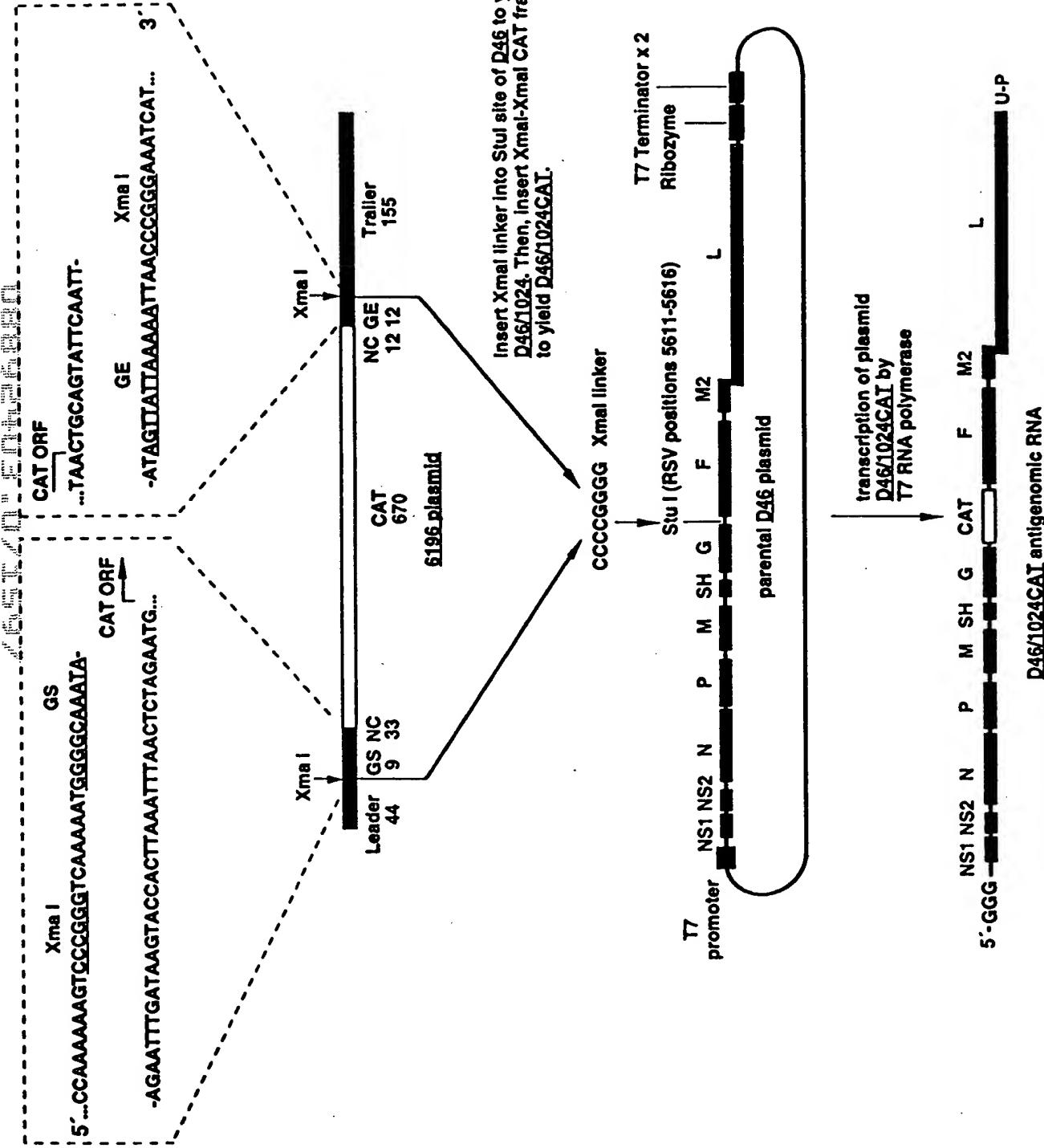
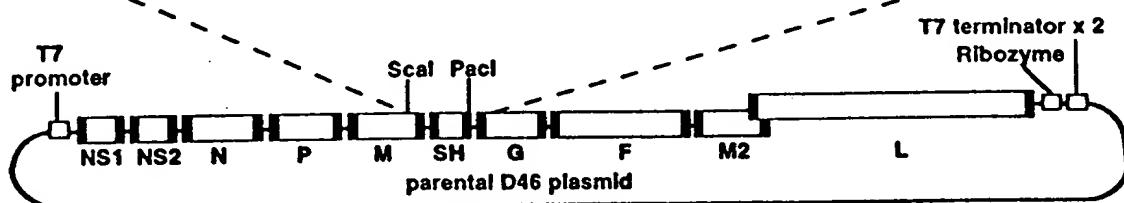
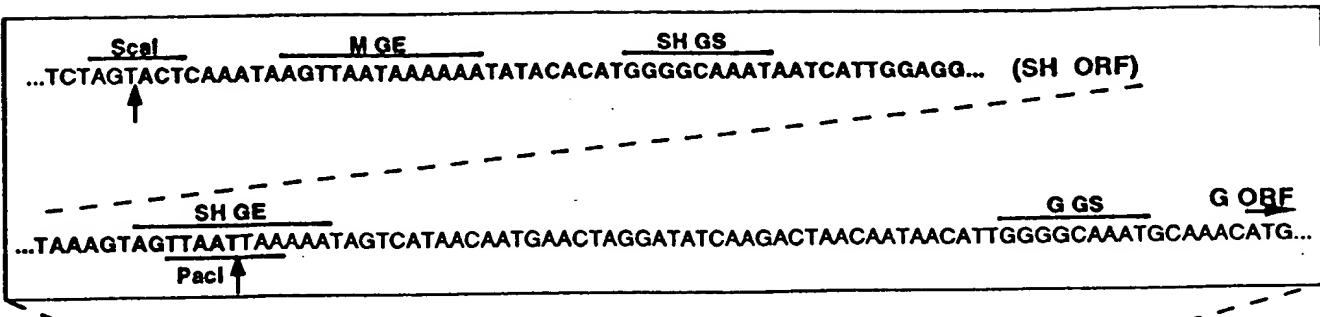
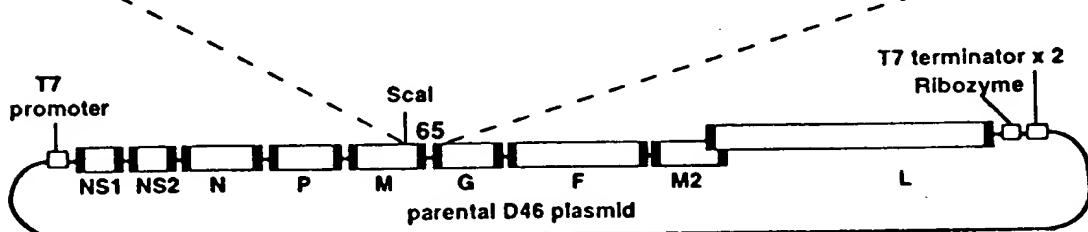
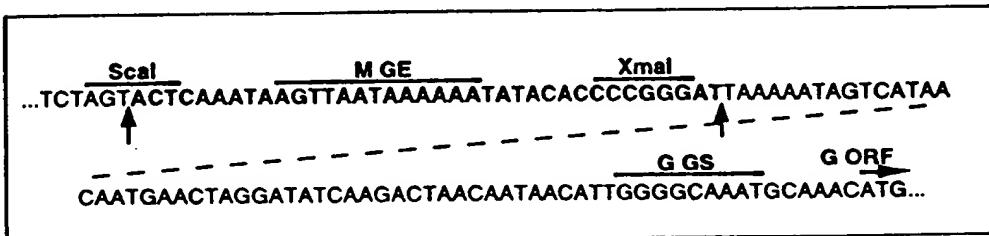


FIG. 6



replace Scal - Pael fragment of D46
with a short synthetic fragment,
thereby deleting the SH gene



transcription of
plasmid D46/6368 by
T7 RNA polymerase

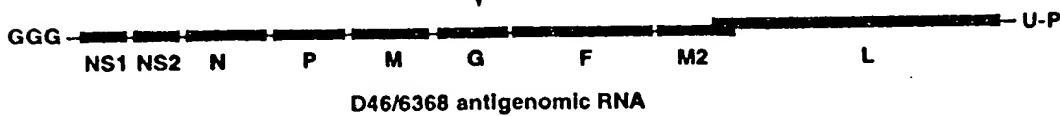


FIG. 7

200 400 600 800 1000

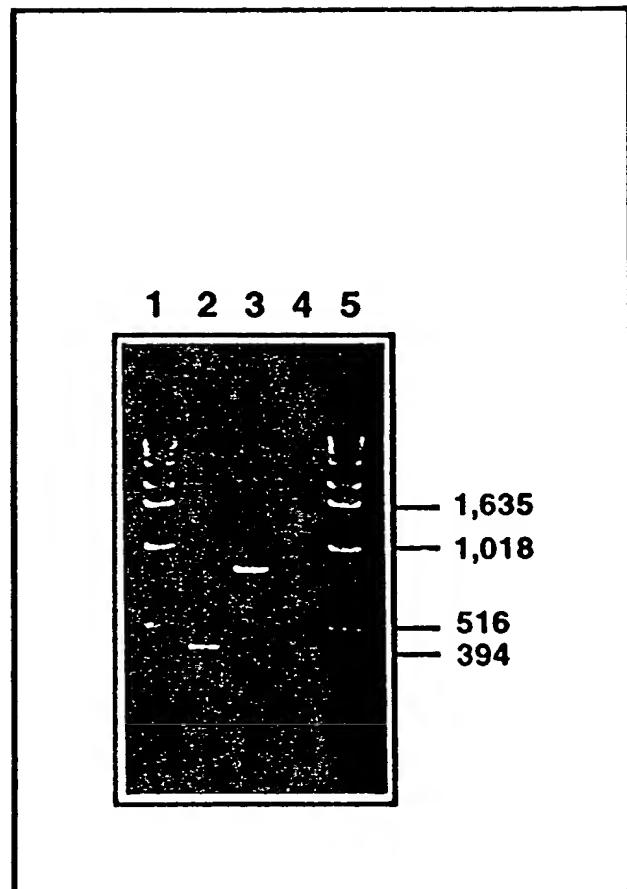
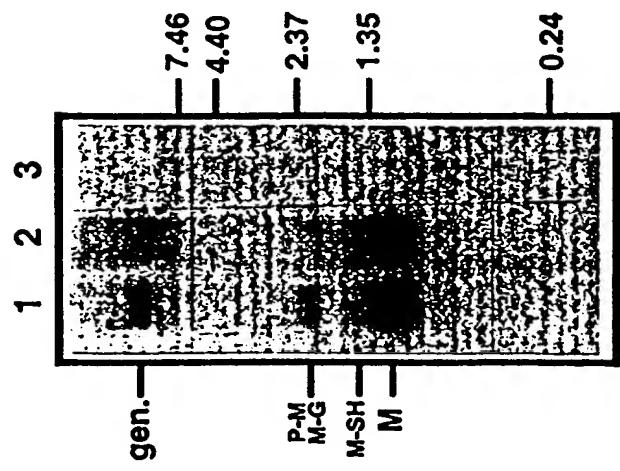


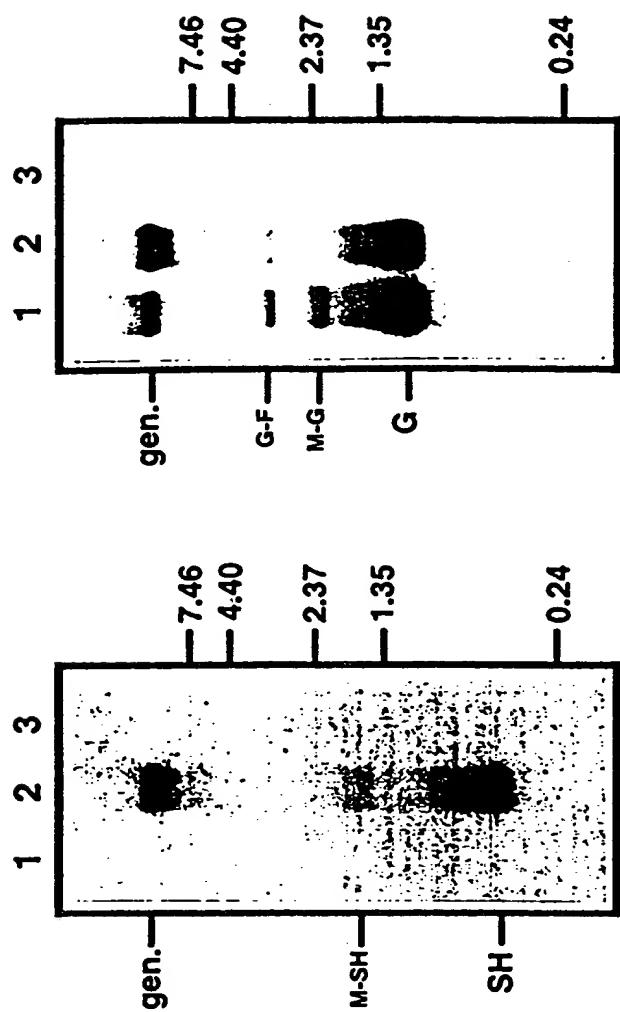
FIG. 8

SH gen. L D SH gen. L D

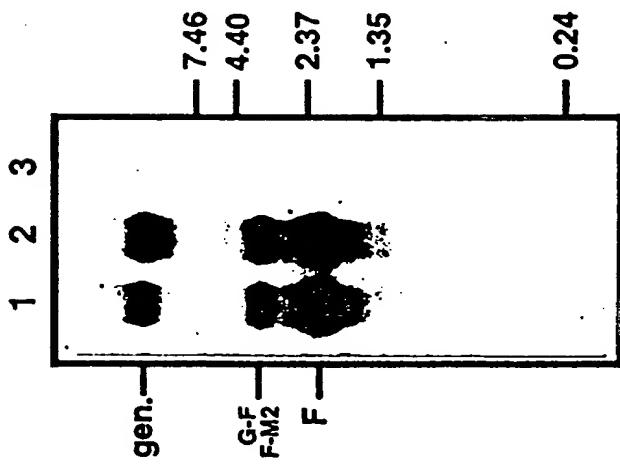
M



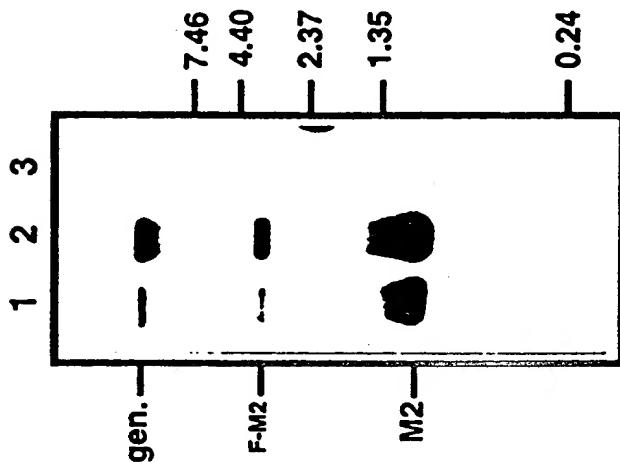
G



F



M2



L

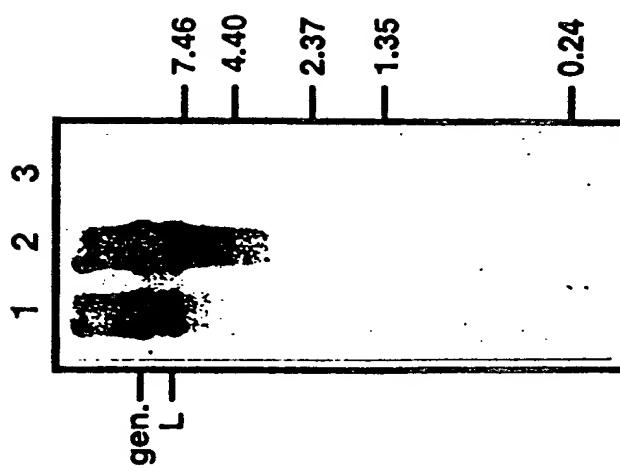


FIG. 9

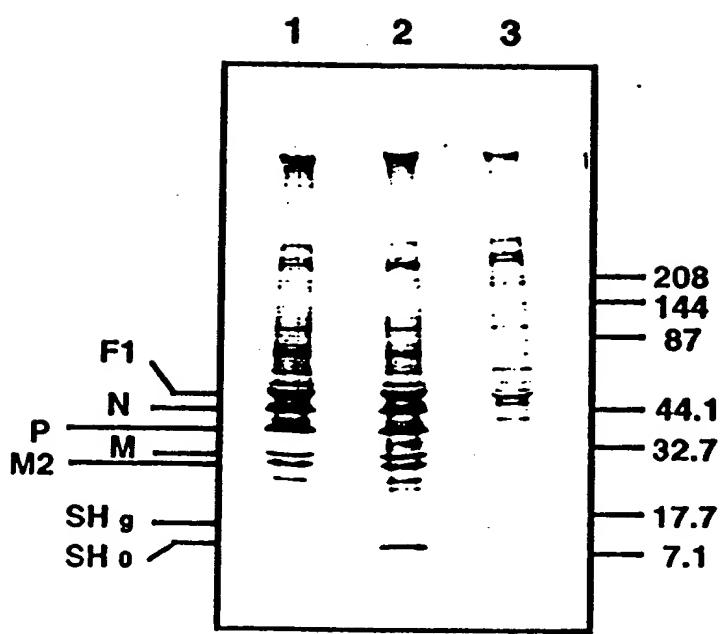


FIG. 10

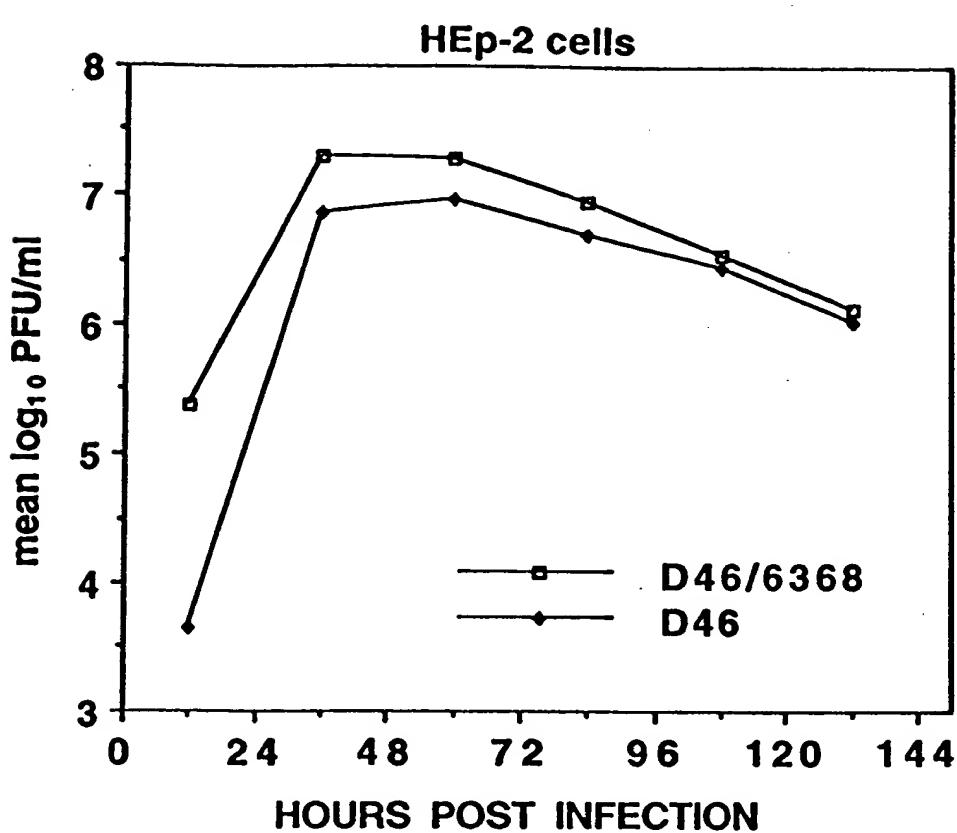


FIG. 11

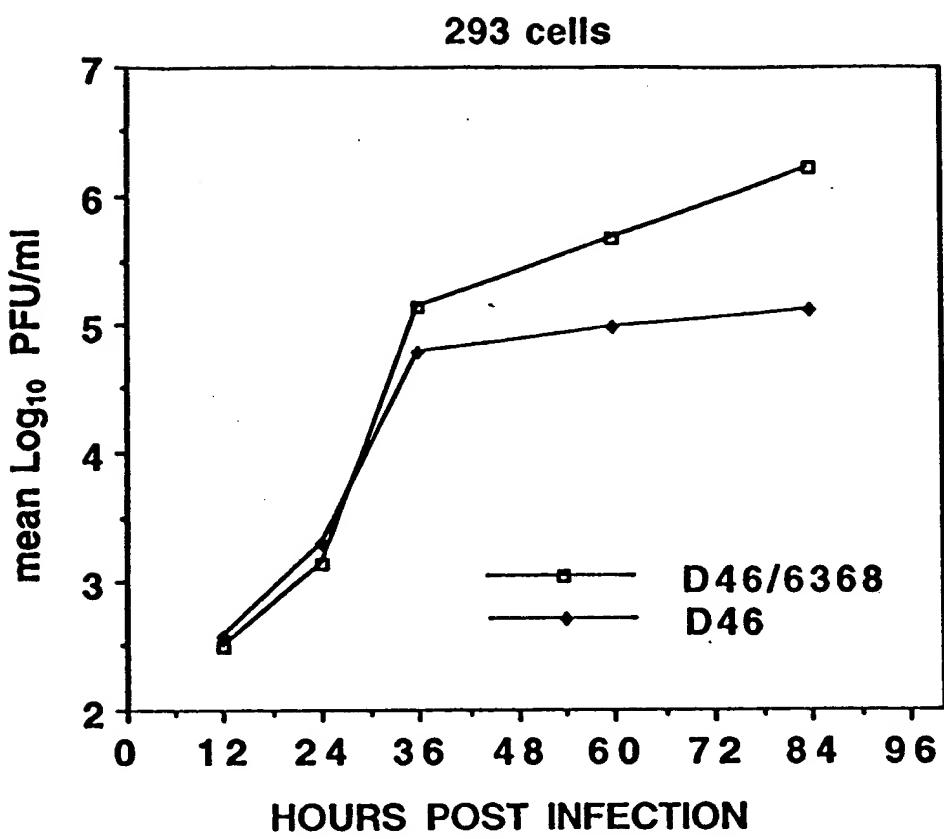


FIG. 12

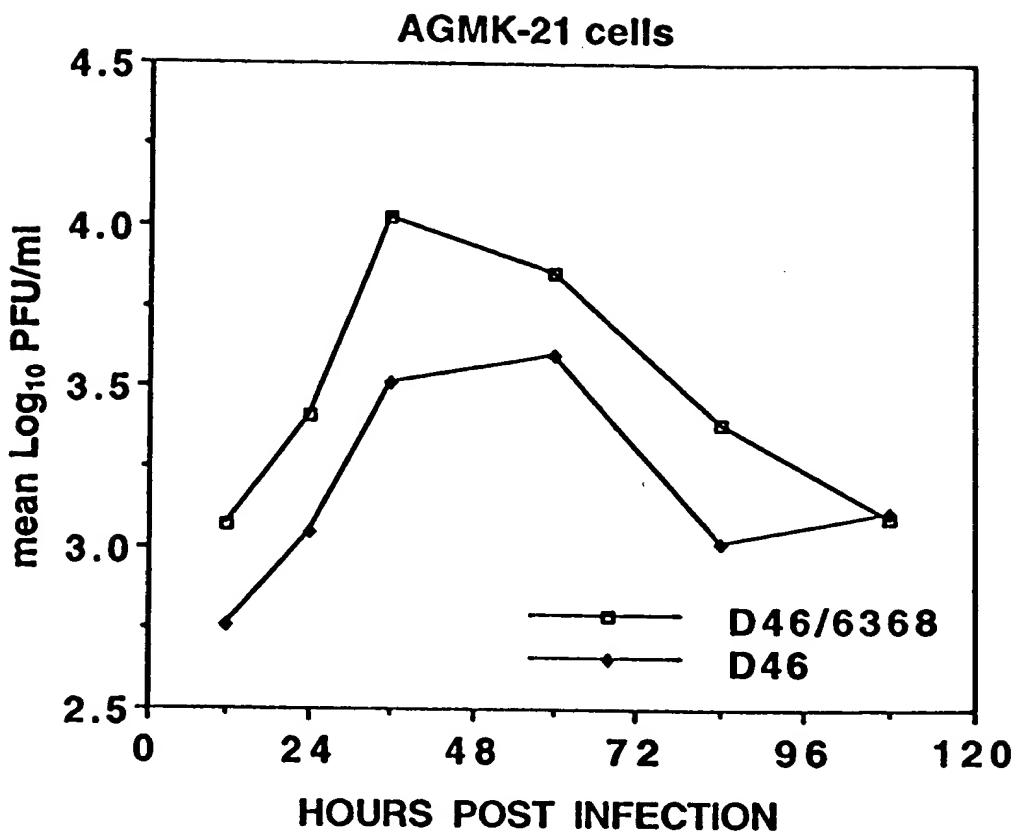


FIG. 13

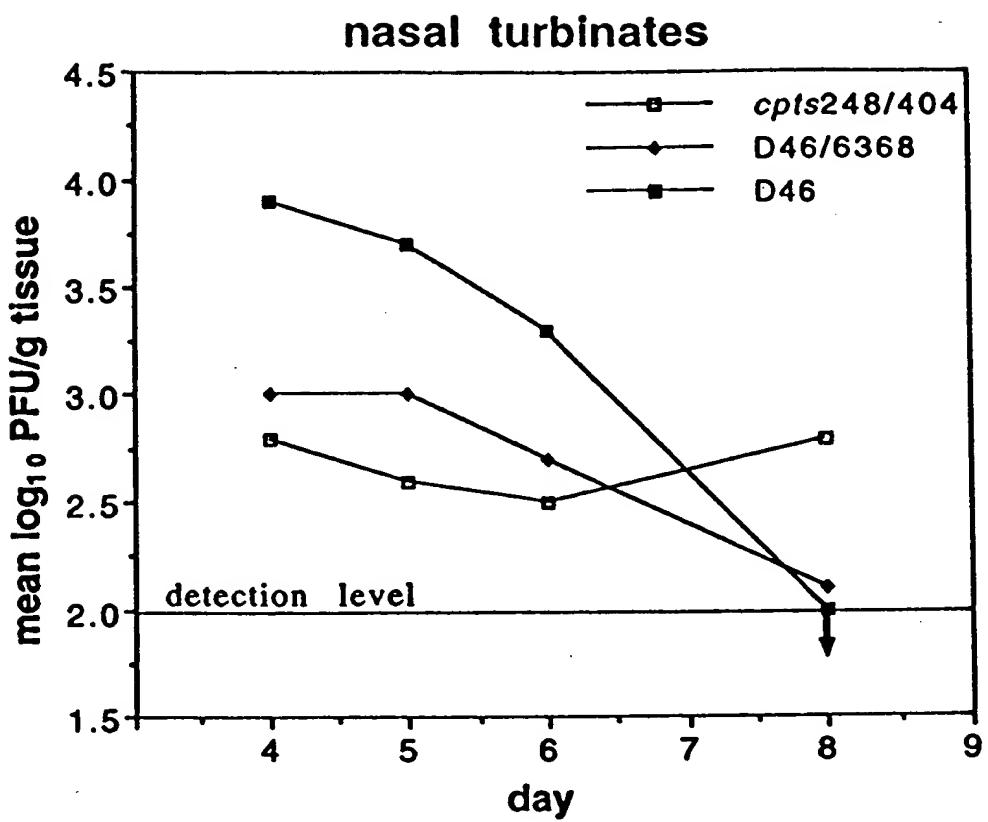


FIG. 14

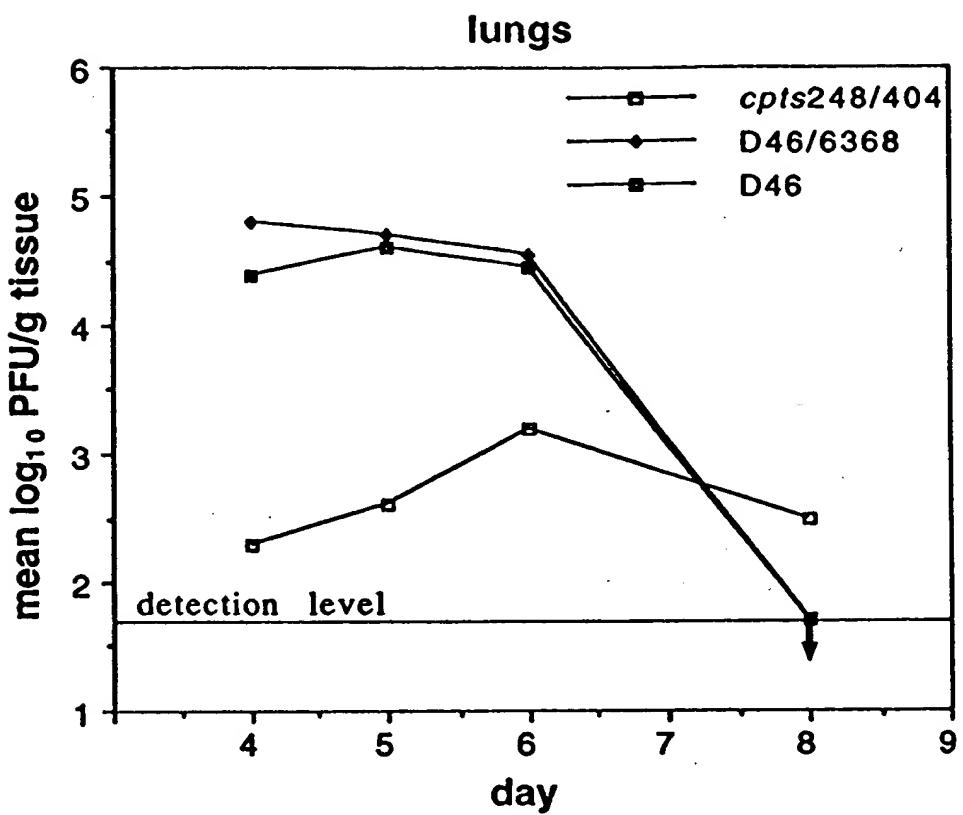


FIG. 15

The SH-minus mutant has a steeper gradient of polar transcription

Relative mRNA abundance: SH-minus / wild type

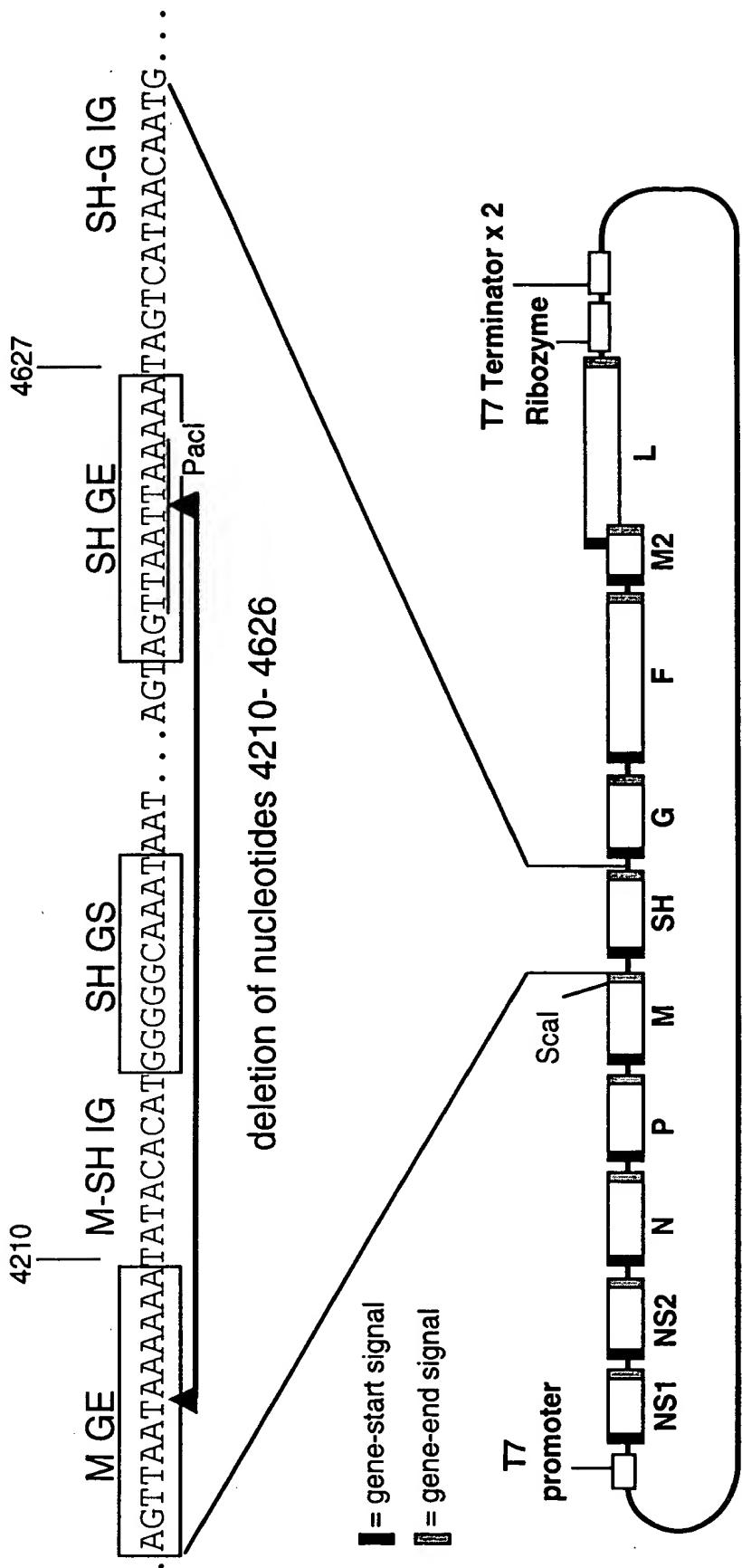
M	G	F	M2	L
1.1	1.3	0.61	0.32	0.17

Positions of genes in 3'- 5' map

WT: 3'- M - SH - G - F - M2 - L
 5 6 7 10

SH-minus: 3'- M - - - G - F - M2 - L
 5 9

FIG. 16



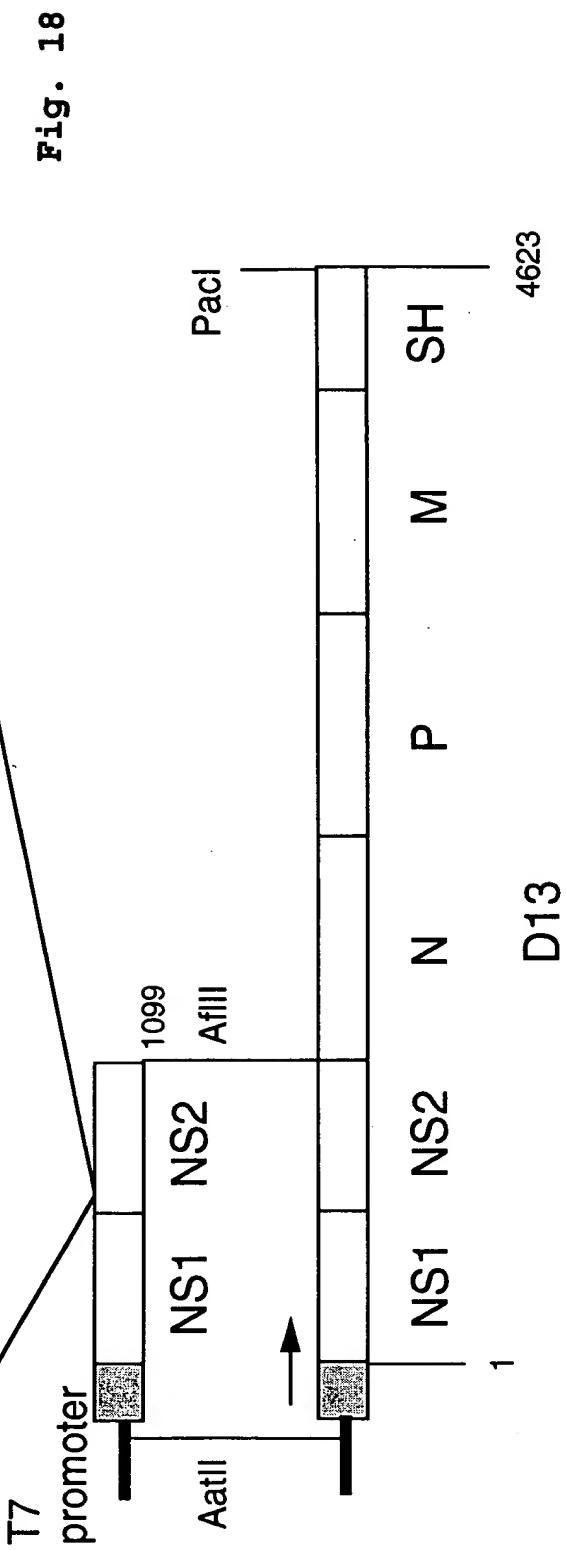
D46/6340HEK plasmid encoding the RSV antigenome with SH deletion (underlined)

Deletion of the complete SH gene.

Note:- the intergenic regions which remain were unaltered in length, and no heterologous sequence was added

Fig. 17

	ter	ter	<u>XbaI</u>
	A	A	C
ATG	AGA	CCG	TTC
M	R	P	L
18			S
			L
			E
			T
			I
			26



Insertion of two tandem translational stop codons into the NS2 translational open reading frame to ablate expression of the encoded protein

Growth Curve of NS2 Knockout Viruses

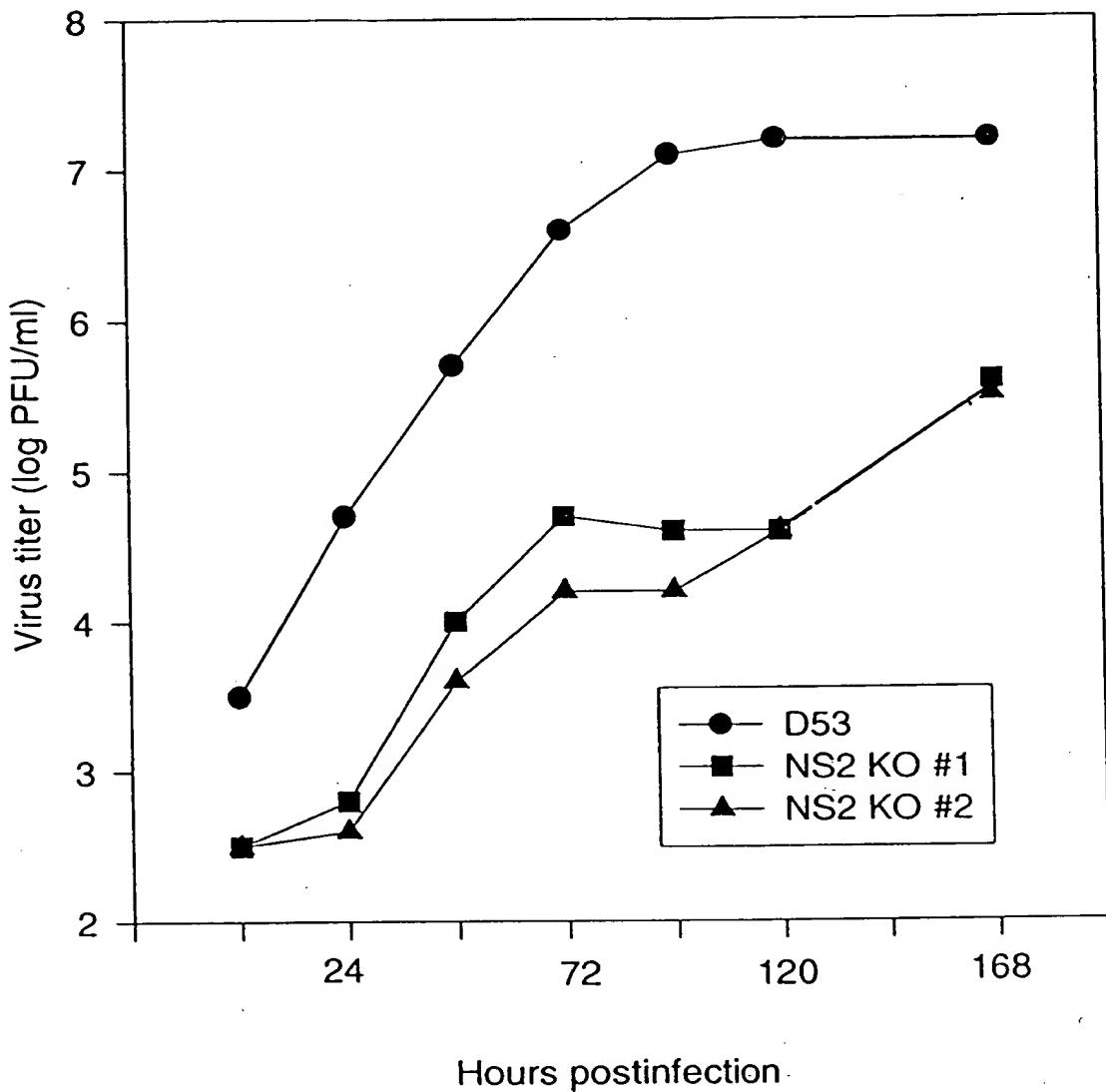
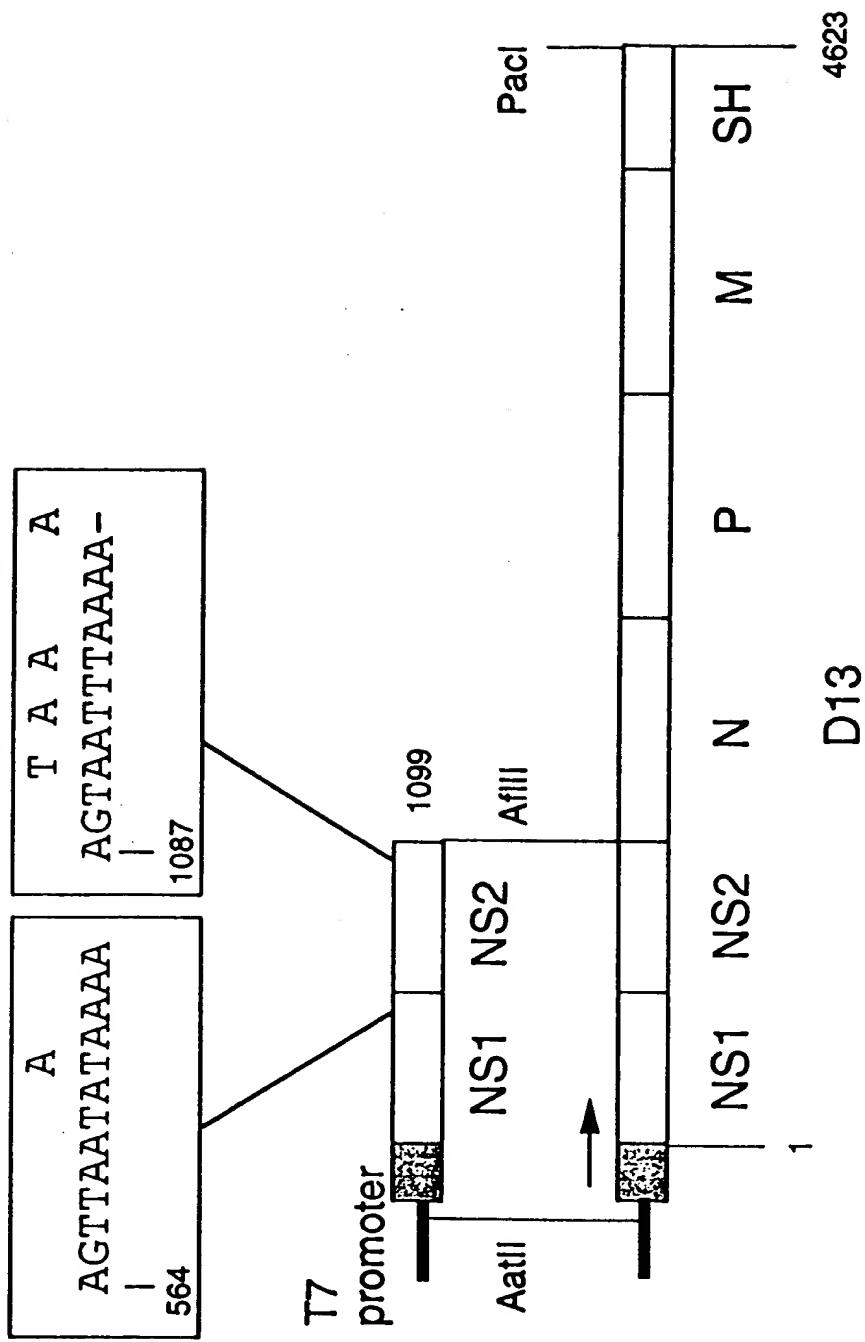


Fig. 19

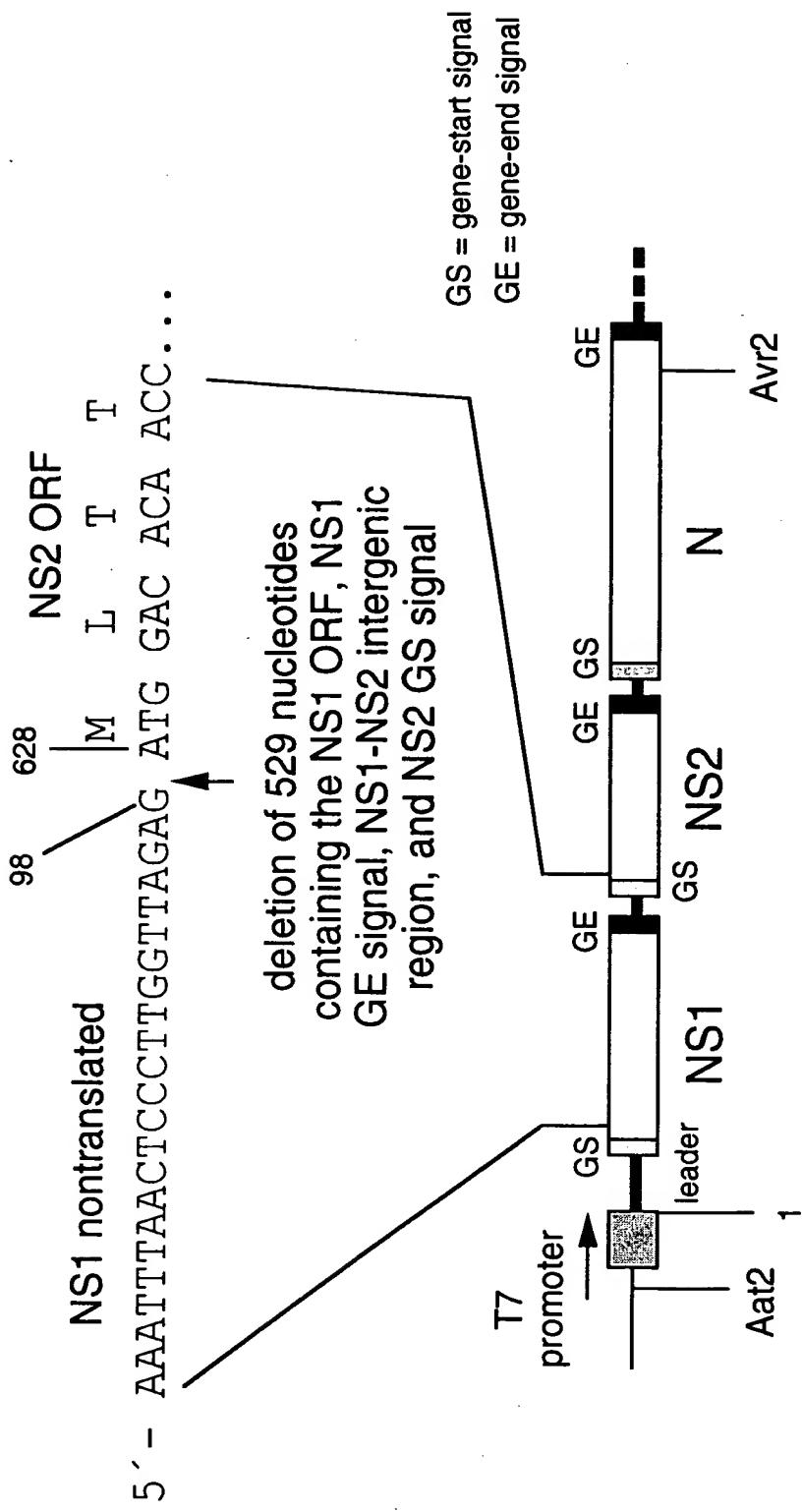
Fig. 20

NS1 Gene End signal NS2 Gene End signal



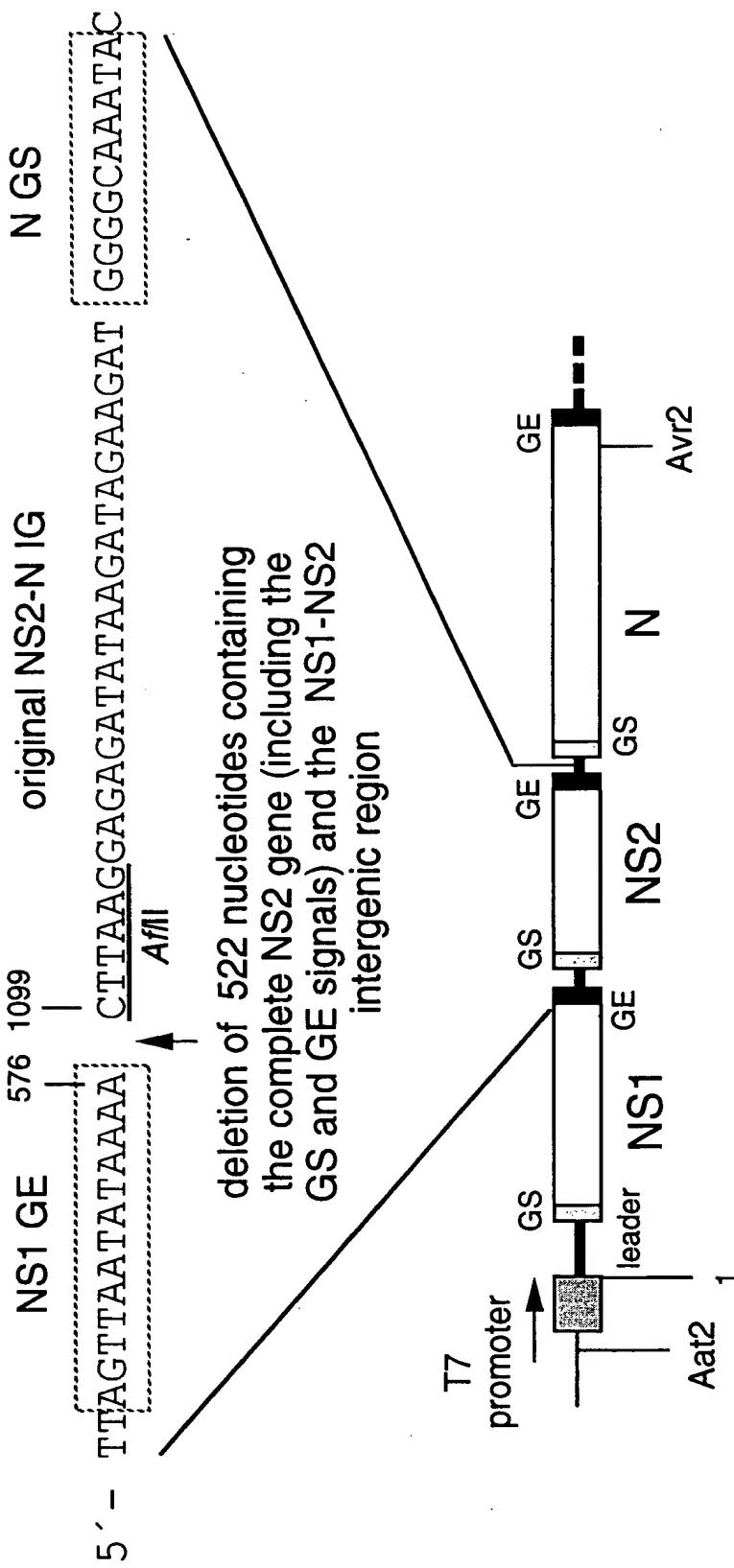
Modification of the Gene End (GE) signals of the NS1 and NS2 genes.

Fig. 21



Deletion of the NS1 gene. The deletion (arrow) begins immediately upstream of the NS1 ATG and extends to immediately upstream of the NS2 ATG.
Note: only the first three genes of the cDNA insert of plasmid D13 are shown.
Numbering is from the first nucleotide of the leader region.

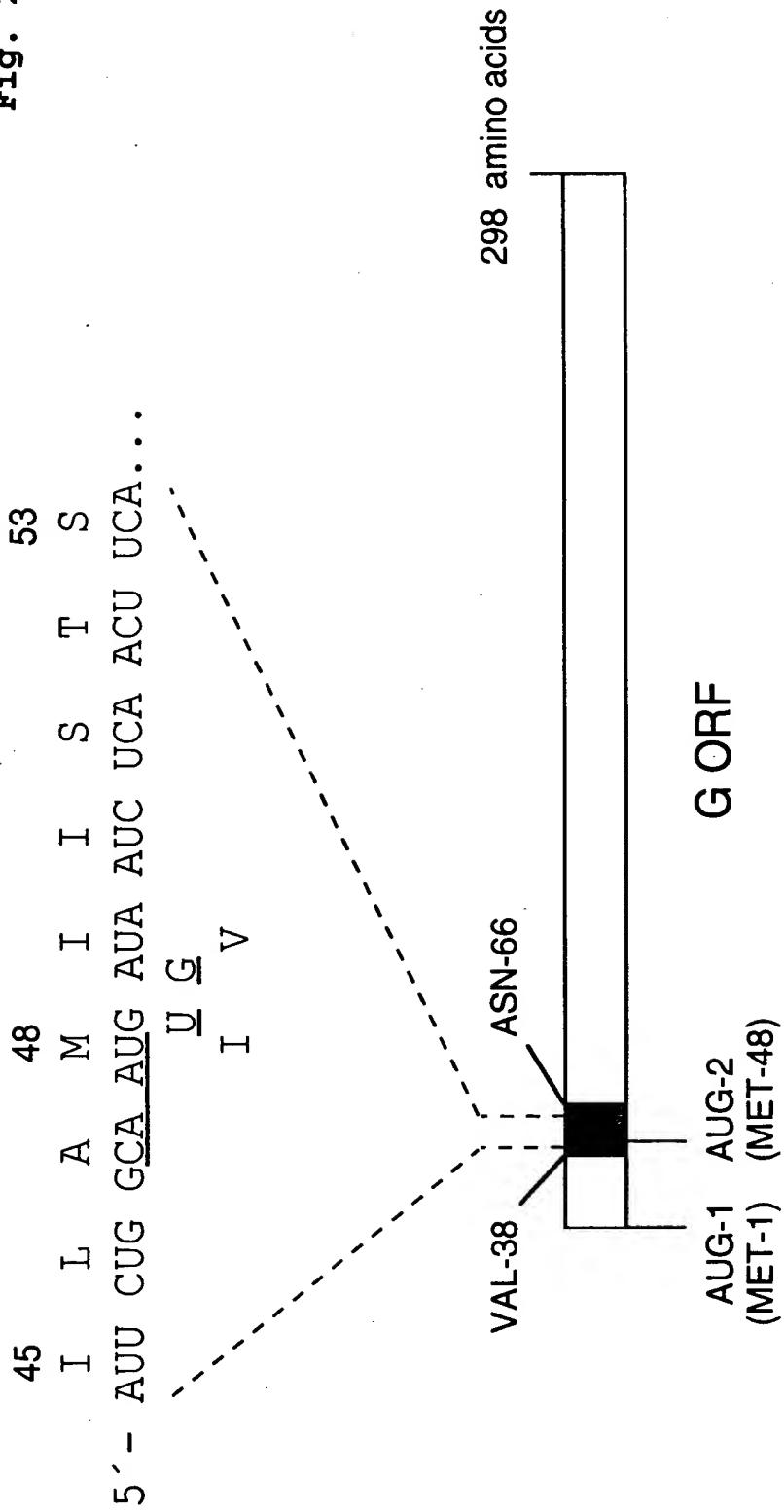
5' - TTAGTTAATAAAA



Deletion of the NS2 gene. The deletion (arrow) begins after the NS1 gene and extends to immediately after the NS2 gene.
Note that only the first three genes of the cDNA insert of plasmid D13 are shown.

Fig. 22

Fig. 23



Ablation of the secreted form of the G protein by mutation of its translational start site. The open rectangle illustrates the G ORF, with the hydrophobic signal-anchor portion filled in. An *M₁* site created by the mutation is indicated by underlining.

Growth Curve of Membrane G Mutants

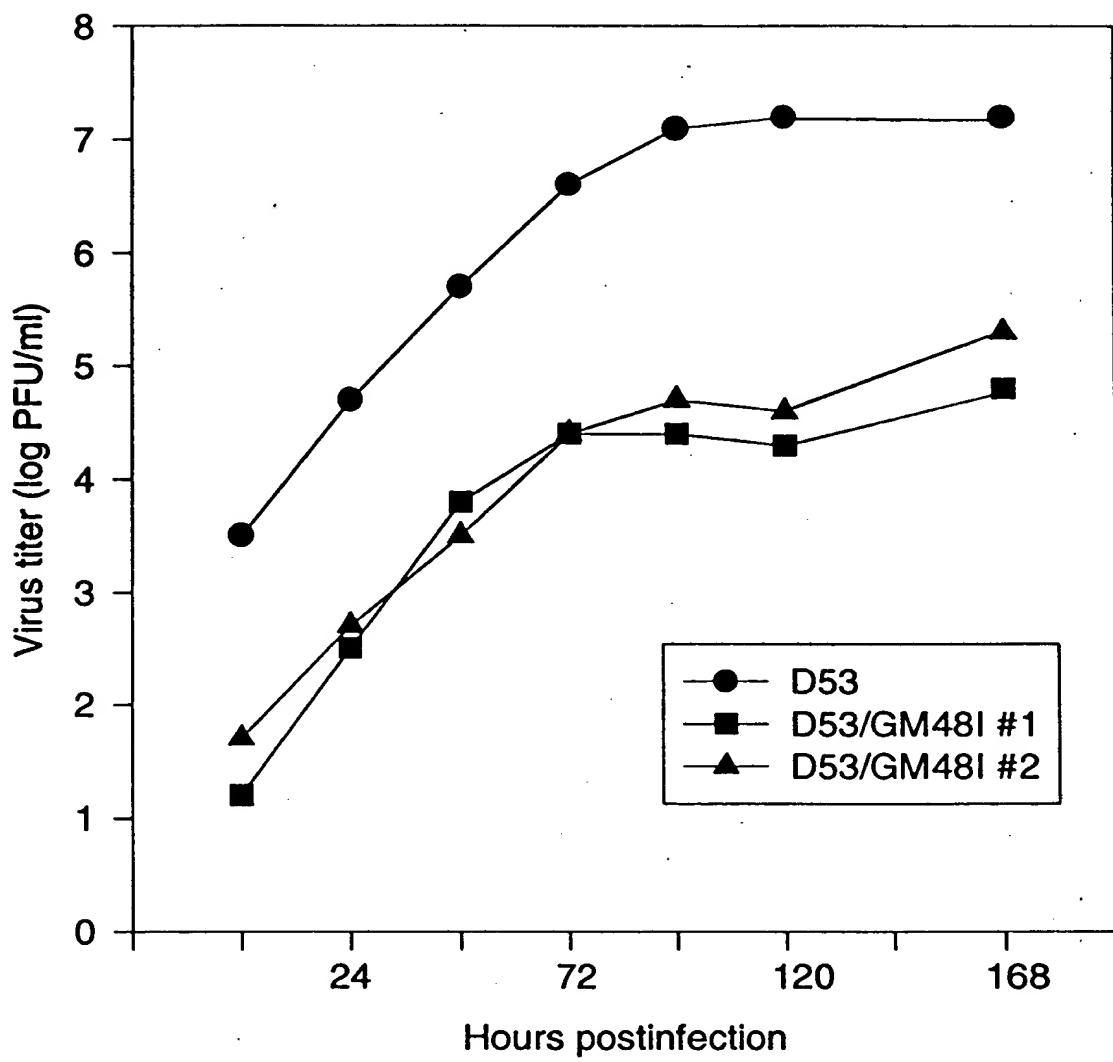


Fig. 24